How Great Wings Can Look Alike

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nectin-1, nectin-2–nectin-2, or nectin-3–nectin-3 binding.

In the mouse inner ear, nectins are expressed in a checkerboard pattern: nectin-1 in hair cells, nectin-3 in supporting cells, and nectin-2 in both. Because each hair cell is surrounded by many supporting cells, heterotypic adhesion between nectin-1 and nectin-3 joins hair cells and supporting cells (see the figure). To test the biological function of differential nectin expression, Togashi *et al*. examined cell arrangements in mice lacking each nectin. Without heterophilic adhesion between nectin-1 and nectin-3, the checkerboard pattern of hair cells was disrupted, leading to homophilic contacts between the same type of cells—for example, the authors observed nectin-1–mediated contact between hair cells in *nectin-3* mutants. It remains unclear whether this affects hearing. Togashi *et al*. also demonstrated that nectins are sufficient for cellular patterning in vitro. When they created an artificial boundary between cultured cells expressing nectin-1 or nectin-3, the cells rearranged at the border to form a mosaic pattern resembling the checkerboard pattern of cells in the inner ear.

Genetic analysis in the mouse is complicated by the presence of four nectins, which may have redundant functions. Thus, mice lacking nectin-1, nectin-2, or nectin-3 individually survive. However, as observed by Togashi *et al*. in the ear, their absence causes defects in cell-cell adhesion in other tissues. In the mouse eye, pigment and nonpigment cells both express nectin-1, -2, and -3. Nectin-1 and nectin-3 mediate heterophilic adhesion between these cell layers, and deleting the gene for either of these nectins causes the cell layers to detach from each other, leading to severe eye defects (*8*). Likewise, in the testis, developing spermatids express nectin-3, whereas nectin-2 is restricted to supporting Sertoli cells. The nectins form heterophilic cell-cell junctions required for spermatid–Sertoli cell adhesion and proper spermatogenesis (*9*). In the developing mouse brain, synapses between mossy fiber terminal axons and CA3 pyramidal cell dendrites require asymmetric distribution of nectin-1 and nectin-3, respectively (*10*). While these defects do not affect neural transmission in the hippocampus, mutations in human nectin-1 sometimes lead to mental retardation.

The findings of Togashi *et al*. are reminiscent of events in fruit fly eyes, another complex sensory structure with a specific arrangement of photoreceptors and supporting cells (*11*). As in mammalian ears, differential cell-cell adhesion shapes this intricate cellular array. Differential expression of E- and N-cadherin regulates cone cell shape. Notably, differential expression of cell surface proteins belonging to another subset of the immunoglobulin superfamily regulates precise arrangement of different types of supporting cells. Family members Kirre and Roughest are expressed in one supporting cell type, whereas Hibris and Sns are present in the other (*12*, *13*). As with nectins, heterophilic protein (and thus cellular) interactions are preferred to maintain the intricate arrangement of cell types.

This family of adhesion proteins also regulates other processes that rely on heterophilic cell interactions in the fly (*14*), including myoblast fusion, axon guidance, and formation of a slit diaphragm–like structure in *Drosophila* nephrocytes. This latter function is striking because the closest human relatives of fly Sns and Kirre are nephrins. Nephrins are mutated in patients with congenital nephrotic syndrome and are required for proper slit diaphragm formation during kidney development. Although nephrins and nectins are not orthologs, these parallels suggest that these and other families of immunoglobulin superfamily proteins may modulate cell sorting and maintain intricate cell patterning in a wide variety of situations.

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EVOLUTION

How Great Wings Can Look Alike

Sean B. Carroll

The *optix* gene controls mimetic wing pattern evolution in *Heliconius* butterflies.

In 1859, English naturalist Henry Walter Bates left Brazil after 11 strenuous, danger-filled, but blissful years of exploring and collecting in the Amazon. Despite all of the privations he had suffered, the self-taught amateur dreaded exchanging a land of "perpetual summer," "endless streams," and "boundless forests" for the "gloomy winters," "murky atmosphere," and "factory chimneys" of England (*1*). But Bates's return home could not have been timed better. Just as he began to sort out his vast collections, Darwin's *On the Origin of Species* appeared and gave him a framework for everything that he had seen in the jungle. Well, almost everything; Bates soon realized that he had noticed some things that had escaped the great Darwin's attention but that could lend support to Darwin's controversial new theory of natural selection.

What Bates discovered were harmless, edible insects, particularly butterflies, whose appearance resembled distasteful or noxious species. Bates reasoned that such striking "analogous resemblances" could not be coincidences but must have come about because the imitators gained an advantage by mimicking well-defended species. Bates's hard-earned, intimate familiarity with the variation within species led him to realize that individuals that were better mimics would fare better than poorer imitators that would be culled by predators. Bates's explanation of mimicry as "a most beautiful proof of the theory of natural selection" (*2*, *3*) was quickly embraced by Darwin and his advocates, and the phenomenon has remained of intense interest to ecologists and evolutionary biologists ever since.

During the 150 years since Bates's descriptions, however, researchers have learned nothing about the mechanisms underlying the generation of mimetic patterns. Until now. On page 1137 of this issue, Reed *et al*. (*4*) identify the *optix* gene, a homolog of a *Drosophila* transcription factor involved in eye development (*5*), as the key regulator of the vivid red color patterns that are characteristic of the classic mimetic wing patterns of *Heliconius* butterflies. The discovery is a major advance because it opens the way toward solving the long-standing mystery of how similar patterns can arise in both closely related and largely unrelated species. The findings also represent a major technological breakthrough in gene

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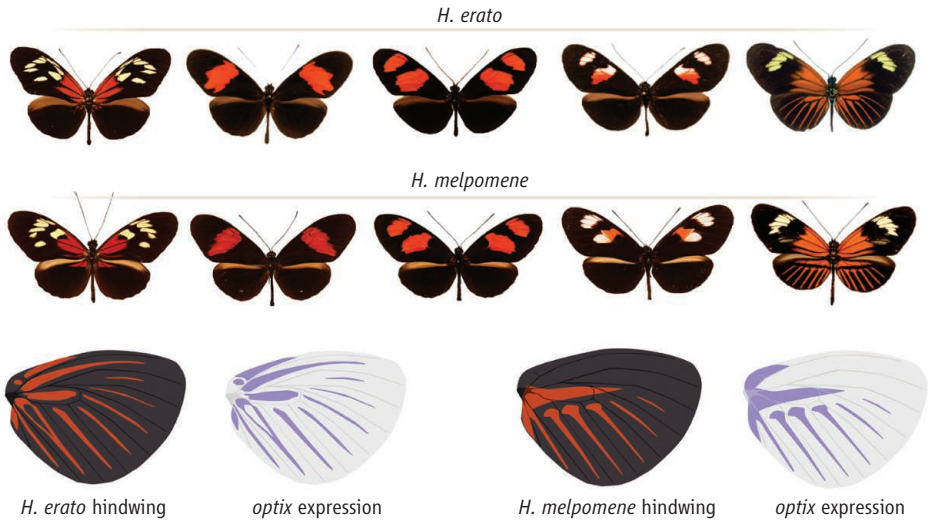
mapping and identification in these experimentally challenging, nonmodel species.

The strikingly beautiful *Heliconius* wing patterns actually present a twist on those first cases of mimicry deciphered by Bates, in that both species of any pair of mimics are distasteful and display warning coloration. It was Fritz Müller, a German-born naturalist who emigrated to Brazil in 1852, who showed that each member of a pair of unpalatable mimics benefited from mimicry and, moreover, that the rarer species gained a greater advantage (*3*, *6*). *Heliconius* butterflies are thus referred to as "Müllerian mimics" to distinguish that phenomenon from "Batesian mimicry," in which a palatable species resembles an unpalatable form.

Heliconius butterflies have attracted extensive interest because of the well-documented co-mimicry that occurs among closely related species in different geographic areas. In the best-studied example, the two species *Heliconius melpomene* and *Heliconius erato* each exhibit great intraspecific variation between regions, but their wing patterns converge upon one another in any given area (see the figure). Such extensive intraspecific morphological diversity, and the convergent evolution of wing patterns in different species under natural selection, has raised the questions of how such diversity is generated during development and how similar patterns evolve in different species (*7*). To answer these questions, investigators must identify the genes involved in wing pattern formation. Crossing experiments performed several decades ago revealed that, despite the apparent complexity of the diverse wing patterns, major differences among geographical races are due to largely Mendelian factors (*8*), but gene identification was technically unapproachable.

Reed *et al*.'s breakthrough required a combination of genomic approaches and some intrepid, long-term fieldwork in Central and South America by members of this extraordinary international research team. Recent studies had narrowed the locus responsible for the red pattern elements on the *H. erato* wing to a 380-kb genomic interval (*9*, *10*). After a systematic survey of all of the genes in the interval revealed a striking correlation between *optix* expression and red patterns in different *H. erato* races and other *Heliconius* species (see the figure), the researchers genotyped wild-caught specimens from hybrid zones in Peru and Costa Rica. These individuals were drawn from populations that had been subjected to thousands of generations of recombination between butterflies with different wing patterns; this enabled the researchers to determine that butterfly wing phenotypes were strongly associated with the genotype at the *optix* locus.



The *optix* gene and butterfly mimicry. (Top) *H. erato* and *H. melpomene* butterflies exhibit great variation in their wing patterns but converge on similar patterns in the same geographic areas (butterflies from the same area are aligned vertically). **(Bottom)** The red patterns on the wings of each species are controlled by the Optix protein. Schematics of the convergent red ray patterns found on the hindwings of one race of *H. erato* and *H. melpomene* are shown next to schematics of *optix* mRNA expression (purple) in developing hindwings. Reed *et al*. (*4*) have shown that the pattern of *optix* expression corresponds to the adult red color patterns.

The link between the *optix* gene and the highly variable red patterns in these butterflies raised the question of the nature of the functional variation at the *optix* locus. The researchers found no coding differences in the Optix protein sequences between highly divergent races of the same species, between divergent phenotypes of closely related species, or between similar phenotypes of distantly related species. Instead, they attributed the functional differences at the *optix* locus to noncoding cis-regulatory sequences. This makes perfect sense in light of the observed differences in the spatial regulation of *optix* messenger RNA (mRNA) expression in different races and species, and what we know about the central role of cis-regulatory mutations in morphological evolution, particularly for changes involving genes with multiple roles (pleiotropic genes), such as *optix*. Research has shown that mutations in the discrete, modular enhancers of pleiotropic genes alter the spatial expression of a gene and the morphology of one body part without altering gene expression or morphology in other tissues, or altering the biochemical activity of the gene product; in general, both kinds of changes would be deleterious (*11*–*13*).

The precise identification of the functionally relevant mutations at the *optix* locus remains to be determined. In addition, we do not yet know the origins of the different *optix* alleles in different races and species. It is of fundamental interest to understand whether similar patterns in different races or species are due to the presence of the same alleles or the consequence of independently derived functional mutations. Thanks to the identification of *optix*, the resolution of this question is now within reach.

In *The Naturalist on the River Amazons,* Bates's riveting narrative of his journey, the explorer waxed poetic about the meaning of the great diversity of butterfly wing patterns he had observed: "It may be said, therefore, that on these expanded membranes nature writes, as on a tablet, the story of the modification of species" (*1*). Those tablets and stories are now being decrypted at their most fundamental level. Bates would be thrilled beyond words.

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